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THE CULTIVATION OF AN ACID-FAST BACILLUS FROM A RAT SUFFERING WITH RAT LEPROSY (A PRELIMINARY REPORT).

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Since the cultivation of the *Bacillus lepra* in symbiosis with amœbas and the cholera vibrio,[1] I have attempted to cultivate by the same method the acid-fast bacillus found in the lesions of rats suffering with rat leprosy.

The amœbas used in these experiments were isolated from the intestines of a guinea pig and grown in symbiosis with cholera vibrio.

The method of isolating and growing in "pure mixed" culture was that of Musgrave and Clegg.[2]

Five-day old cultures of this "pure mixed" culture of amœbas and cholera vibrios were inoculated with blood taken from the heart of a rat. This rat was the descendant of 1 of 12 rats inoculated with rat leprosy by Passed Asst. Surg. McCoy in 1909. It was allowed to live in continuous contact with these inoculated rats and subsequently developed the disease. It showed advanced lesions of the disease known as rat leprosy. Smears from the lesions, as well as from the heart blood, showed numerous acid-fast bacilli.

The inoculated tubes were placed in the incubator at 37° C. and allowed to remain at this temperature for six days. Smear preparations from the inoculated tubes made at the end of this period showed many changes in the morphology of the acid-fast bacilli. The bacilli became longer, with the beads well marked, while a budding or branching process was also observed. The bacilli then became much smaller than those observed in the tissues of rats suffering with rat leprosy, in some cases being almost of a coccoid form. The number of bacilli present showed an increase over the number originally found in the rats' heart blood.

Transplants were then made to tubes of cholera vibrio with amœbas 5 days old, and placed at the same temperature, examinations of smears made at periods of from 6 to 13 days showed on a number of the sides evidences of multiplication of the microorganism. With the succeeding subcultures in 5-day old cholera vibrio with amœba tubes, the acid-fast bacilli increased proportionally in numbers.

In the ninth generation attempts were made to separate and to grow the acid-fast bacilli in pure culture. This was done by heating the mixed cultures of amœbas, cholera vibrios, and the acid-fast bacilli for 30 minutes at a temperature of 62° C. This temperature was sufficient to kill the cholera vibrios and the amœbas.

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[1] Clegg, M. T., 1909. The Cultivation of the Leprosy Bacillus. Philippine Journal of Science, sec. B., 1909, vol. 4, No. 77, p. 403.

Currie, D. H., Brinckerhoff, W. R., and Hollmann, H. T., 1910. On the Cultivation of the Bacillus of Leprosy by the Method of Clegg. Public Health Reports, United States Public Health and Marine-Hospital Service, Aug. 26, 1910.

[2] Musgrave, W. E., and Clegg, M. T., Amebas: Their Cultivation and Etiological Significance. Pub. P. I. Bur. Sci. Biolog. Lab. (1904).

Cultures were then made from the heated mixture on glycerin agar in tubes and incubated as before.

After a period of 21 days distinct colonies were observed on the media. These colonies were raised, had smooth irregular borders, and were pale yellow in color.

Stained preparations made from these colonies showed an acid-fast bacillus. Subcultures grew well on glycerin agar with a smooth raised, creamy-appearing growth of a lemon-yellow color, which was found to be easily removed from the media with the oese. On plain and nutrient agar, the growth was of a lighter lemon-yellow color, but not so profuse a growth as on glycerin agar.

ANIMAL INOCULATIONS.

Eight white rats were inoculated subcutaneously with a suspension in saline solution of the third generation of pure culture, each rat receiving an amount of the suspension equivalent to five oesen of the pure culture. One of these rats died on the thirty-seventh day after the inoculation. The autopsy revealed an inflammatory reaction around the site of the inoculation, in which acid-fast bacilli were found.

Another died on the forty-eighth day. The autopsy revealed an abscess at the site of inoculation, and minute areas of consolidation in the lungs. Smears made from the contents of the abscess contained acid-fast bacilli.

The remaining six rats were chloroformed on the one hundred and sixteenth day. The autopsies of these six rats revealed ulcerations at the site of the inoculations in which acid-fast bacilli were found.

In two there was consolidation of the lungs. Smears made from one of these consolidated lungs contained acid-fast bacilli. In one rat smears from a nasal lesion contained acid-fast bacilli.

Three white rats were inoculated intraperitoneally with the same amount of this suspension. They were chloroformed on the one hundred and sixteenth day. The autopsies revealed no apparent pathological change, but smears made from the spleens of two of these rats showed acid-fast bacilli.

Two guinea pigs were inoculated with the same amount of the suspension, one subcutaneously, the other intraperitoneally. They were chloroformed on the one hundred and ninety-seventh day. The autopsies revealed no pathological changes, nor were acid-fast bacilli found.

SUMMARY.

I have succeeded in cultivating in pure culture an acid-fast bacillus from a rat suffering with rat leprosy by the method described by Clegg in the cultivation of the human lepra bacillus.

Of the eight rats inoculated with the pure culture subcutaneously acid-fast bacilli were found in all, in the lesions of the skin of eight, in the lungs of two, and in the nasal discharge of one.

Of the three rats inoculated intraperitoneally no lesions of the skin nor of the internal organs were found, but the splenic pulp in two of them contained acid-fast bacilli.